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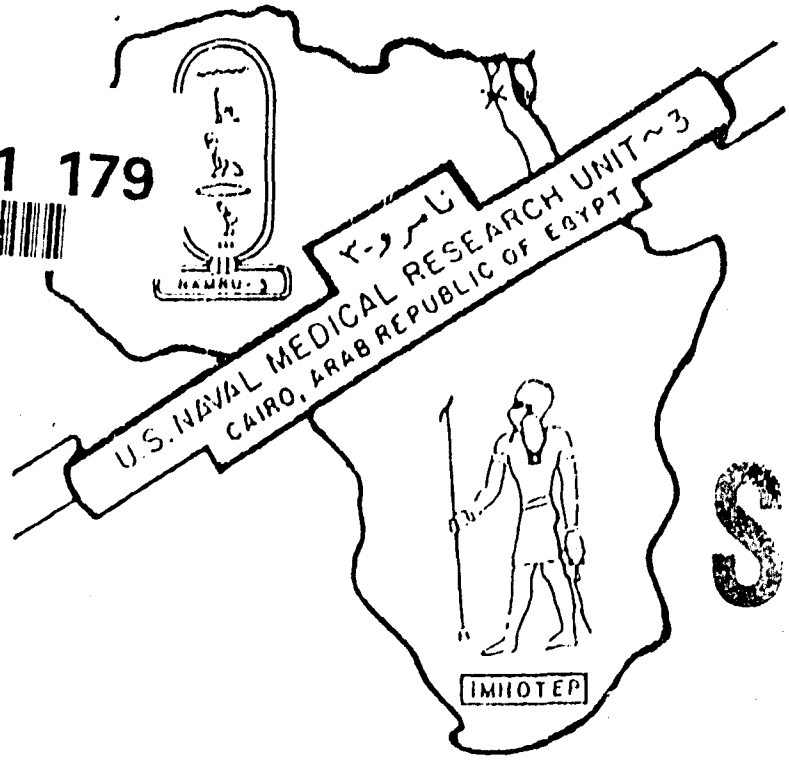

PUBLICATION REPORT

1602

84/89-00

SEROLOGICAL EVIDENCE OF DENGUE FEVER AMONG REFUGEES, HARGEYSA, SOMALIA

BY

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FPO NEW YORK 09527

Journal of Medical Virology 29:79–81 (1989)

Serological Evidence of Dengue Fever Among Refugees, Hargeysa, Somalia

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Epidemics of a malaria-like illness affected several thousand residents of the Dam Camp, a refugee camp near Hargeysa in Somalia, during 1985, 1986, and 1987. The disease was characterized by fever, chills, sweats, headache, back and joint pains for as long as 10 days in some patients. Blood smears from acutely ill patients were negative for malaria. Of 28 acute and 10 convalescent sera tested by the indirect fluorescent antibody (IFA) and by the hemagglutination inhibition (HI) tests, all were negative for antibody to Rift Valley fever, Crimean-Congo hemorrhagic fever, Sindbis, Chikungunya, yellow fever, and Zika viruses. However, antibody reactive to dengue 2 virus was detected by the IFA test in 39% (15/38), and 11 of 29 (38%) of the same sera were antibody positive by the HI test. Also, IgG antibody reactive to dengue 2 was demonstrated in 60% (17/28) of the same sera by the enzyme immunoassay (EIA), and 14% (4/28) were positive for IgM antibody. Of ten patients for which acute and convalescent sera were available, two developed four fold or greater rises in antibody titer evidencing infection These data suggested that dengue virus may have been the cause of the epidemic among the Dam Camp refugees.

KEY WORDS: Dengue, ELISA, IgM antibody

INTRODUCTION

The first documented isolation of dengue virus in Africa occurred between 1964 and 1968 in Nigeria [Carey et al., 1971], and thereafter in 1972–1975, dengue 1 (DEN-1) and dengue 2 (DEN-2) viruses were frequently isolated from humans [Fagbami and Fabiyi, 1976]. Of 32 strains of dengue virus isolated from patients in Nigeria, 18 were identified as DEN-1 and 14 as DEN-2 [Carey et al., 1971]. In Upper Volta, six strains of DEN-2 virus were isolated from 80 patients in 1982, and 30% of these patients had IgM antibody to DEN-2 virus [Gonzales et al., 1985]. In Kenya, seven strains of DEN-2 virus were isolated in 1982, and a similar strain was isolated from a Canadian tourist [Johnson et al., 1982]. In Port Sudan, 17 strains of DEN-2 virus were isolated during 1984, as well as one strain of DEN-1 [Saleh et al., 1985]. In 1983, three expatriates in Somalia had serologic evidence of flavivirus infection with a rise in IgM antibody titer to DEN-2 virus [Saleh et al., 1985]. However, in 1984 a limited follow-up survey showed no evidence of endemic dengue virus transmission [Saleh et al., 1985] in this area.

HISTORY OF THE DISEASE IN THE DAM CAMP

In 1985, an epidemic of malaria-like illness occurred in Dam Camp, a refugee camp near Hargeysa (Fig. 1), Somalia. It affected several thousand residents, but no deaths were reported. In 1986, another outbreak occurred in the same camp. It lasted about 2 months, and no deaths were reported. The disease manifestations were fever, as high as 39°C, chills, sweats, headache, back pain, and joint pain. The disease was recognized by the camp inhabitants as the "bone breaking sickness" "JeJeebiye." No rash, no hepatomegaly or splenomegaly, or signs of meningism were recorded, the disease lasted for about 10 days with gradual reduction in severity. The disease spread throughout the camp affecting both sexes but adults more than children. A similar outbreak occurred in the same camp during late 1987 but was less extensive. In addition, during

Accepted for publication June 4, 1989.

Address reprint requests to Research Publications Branch, NAMRU-3, FPO New York, NY 09527-1600.

This work was supported by the Naval Medical Research and Development Command, NMC, NCR, Bethesda, MD 20814, Work Units No. 3M162770.A870.AR.322 and 3M162770A870.AQ.320 The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or as reflecting the views of the Navy Department, Department of Defense, the U.S. Government, or the Ministry of Health of Somalia

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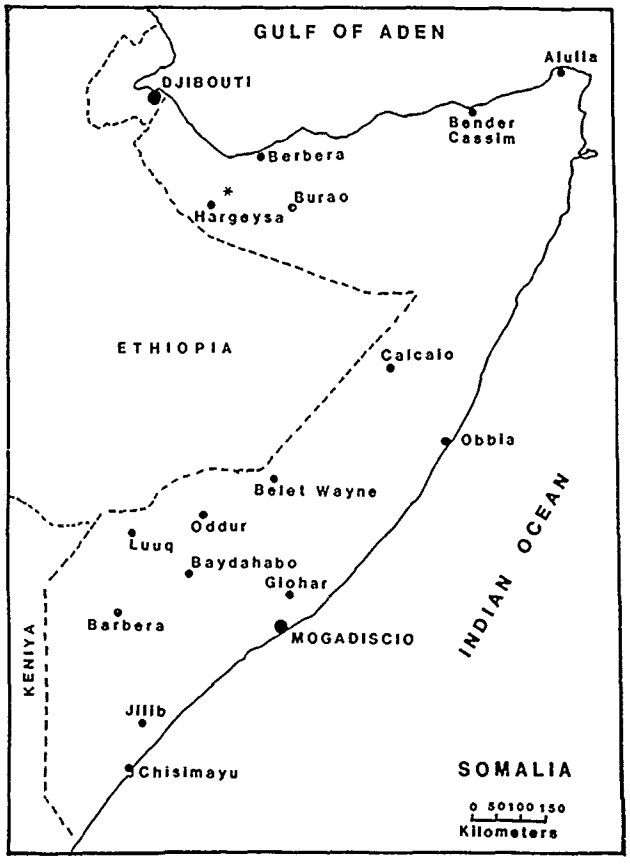

Fig. 1. Map of Somalia. *, site of epidemic.

the same year, a similar illness reached epidemic proportions in the town of Hargeysa (Fig. 1).

During 1987 outbreak, blood samples were collected by the Refugees Health Unit physician from some of the patients and were referred by the Ministry of Health in Somalia to the U.S. Naval Medical Research Unit No. 3 (NAMRU-3), Cairo, Egypt, through the World Health Organization representatives in Mogadishu and Eastern Mediterranean Regional Office, Alexandria, Egypt, for diagnostic testing.

This report describes studies conducted to determine the etiology of the disease observed among the refugees of the Dam Camp in Somalia.

MATERIALS AND METHODS

Materials

A total of 28 sera were collected from patients who reportedly had acute illnesses. Additional sera were obtained from ten of these 28 patients 10 days later. Of the 28 acute sera, four were from patients that were febrile at the time of blood collection.

Methods

Sera were initially screened by the indirect fluorescent antibody (IFA) and hemagglutination inhibition (HI) assays for antibody to Crimean-Congo hemorrhagic fever (CCHF), Rift Valley fever (RVF), dengue type-2 (DEN-2), Chikungunya (CHIK), Sindbis (SIN), West Nile (WN), yellow fever (YF), and Zika (ZIKA) viruses. Further testing of sera for evidence of dengue viral infection was done by the enzyme immunoassay (EIA).

TABLE I. Serologic Evidence of Arboviral Infections Among Refugees of Dam Camp in Hargeysa, Somalia*

	Acute (No. pos./ No. tested)		Convalescent (No. pos./ No. tested)	
Antigen	IFA[a]	HI[b]	IFA	HI
Sindbis	ND	0/28	ND	0/10
Chikungunya	ND	0/28	ND	0/10
Dengue 2	12/28	8/21	3/10	3/8
West Nile	1/28	ND	0/10	ND
Yellow fever	0/28	ND	0/10	ND
Rift Valley fever	ND	0/28	ND	0/10
Zika	0/28	ND	0/10	ND
Crimean-Congo hemorrhagic fever	0/28	ND	0/10	ND

*ND = not done.
[a]IFA = indirect fluorescent antibody.
[b]HI = hemagglutination inhibition.

The IFA test [Wulff and Lang, 1975] was performed using composite spot antigen slides provided by the United States Army Medical Research Institute of Infectious Diseases (Fort Detrick, Frederick, Maryland). The HI test [Clarke and Casals, 1958] was performed using Beta-propiolactone (BPL) -inactivated sucrose acetone-extracted mouse brain antigens and cell culture antigens. The EIA was performed for IgG virus-specific antibody as well as for IgM-specific antibody by an IgM capture assay [Burke et al., 1982] using sucrose-acetone-extracted antigens and affinity-purified goat antihuman horseradish peroxidase conjugate (Kirkegaard and Perry, Gaithersburg, Maryland).

Sera were diluted twofold, starting with a 1:10 dilution, and those reacting at ≥1:40 dilution by the IFA and HI tests were considered positive. Sera were tested by EIA at a 1:200 dilution, and those having an OD ≥ greater than or equal to the mean of ten known negative sera plus 3 standard deviations were considered positive Pooled human plasma from dengue vaccinees was used as positive control. Sera from children residing in nonendemic dengue area were used as negative control.

Sera obtained from four febrile patients were inoculated onto mammalian cell cultures, VERO clone E-6 and BHK-21, and intracerebrally into 1–3 day old Swiss mice for virus isolation attempts. Cell cultures and mice were observed for 10 days for cytopathic effect (CPE) and signs of illness, respectively. A blind passage was performed in the same assay systems if CPE was not observed in cells or if mice did not exhibit signs of illness following primary inoculation with sera.

RESULTS

Antibodies to SIN, CHIK, YF, RVF, ZIKA, and CCHF viruses were not detected in any of the sera

TABLE II. Summary of IFA, HI, EIA IgG, and EIA IgM Dengue 2 Antibody Detected Among Dam Camp Refugees in Somalia

Assay	Acute sera No. positive/ No. tested (%)	Convalescent sera No. positive/ No. tested (%)	Total No. positive/ No. tested (%)
IFA[a]	12/28 (43)	3/10 (30)	15/38 (39)
HI[a]	8/21 (38)	3/8 (37)	11/29 (38)
EIA IgG[b]	14/19 (74)	3/9 (33)	17/28 (60)
EIA IgM[b]	3/19 (16)	1/9 (11)	4/28 (14)

[a]Titers ≥40 were considered positive.
[b]Sera screened at 1:200 dilution.

TABLE III. Dengue Antibody Titers of Acute and Convalescent Sera of Two Patients From the Dam Camp in Somalia[a]

	Patient 1		Patient 2	
	Acute serum	Convalescent serum	Acute serum	Convalescent serum
IFA[b]	10	40	10	40
HI[b]	40	160	<20	40
EIA IgM[c]	<200	≥200	<200	<200
EIA IgG[c]	<200	≥200	≥200	≥200

[a]Reciprocal of serum dilution.
[b]Titers ≥40 considered positive.
[c]Tested at 1:200 dilution.

collected from the refugees (Table I). Fifteen (39%) of 38 sera had IFA antibody, and 11 (38%) of 29 had HI antibody to DEN-2 virus. One (3%) of the 38 sera had IF antibody to WN virus. The results of the IFA, HI, and the EIA IgG and IgM antibody tests employing DEN-2 antigen are summarized in Table II. Approximately 40% of the sera were antibody positive by both IFA and HI tests (15/38 and 11/29, respectively). Sixty percent (17/28) of sera had IgG antibody, and 14% (4/28) had IgM antibody reactive to DEN-2 virus.

A second serum sample was available for ten patients, and a fourfold rise, or greater, in antibody titer was demonstrated by the IFA and HI tests in two patients (Table III). The acute serum of one of these patients was negative, but IgM antibody was demonstrable in the convalescent serum. Virus was not isolated from the acute sera of the four febrile patients.

DISCUSSION

The clinical description of the febrile illness recorded during 1987 among the refugees of the "Dam Camp" and the serological results suggest that dengue virus was most likely the etiology of this disease outbreak. In the preceding 2 years, similar but more severe outbreaks had occurred in the same camp. It appears that the same disease affected the refugees for 3 successive years with the last outbreak characterized by mild illness, possibly because of immunity acquired previously. Although dengue virus was not isolated from sera of the four febrile patients, most likely because of inadequate refrigeration of samples during transportation, antibody demonstrated by the EIA, IFA, and HI tests indicated that the patients had been infected by dengue virus. Furthermore, the demonstration of IgM antibody to DEN-2 virus and a fourfold or more rise in antibody titer from acute to convalescent sera in two patients was indicative of recent infection by this virus.

The limited volume of sera precluded the use of neutralization tests required to determine the serotype of dengue virus. While the possibility that results for dengue reflect other flaviviral infections could not be entirely excluded, serological evidence of infections by WN and YF were not demonstrated except in one of the 28 patients studied (Table I). Thus, the clinical description of the illness and the serological results strongly suggest that dengue virus was the most likely cause of the disease outbreak among the Dam Camp refugees.

ACKNOWLEDGMENTS

The authors acknowledge with respect Dr. M. Barzagar, WHO representative in Mogadishu, Somalia and Dr. M. Wahdan, Director, Disease Prevention and Control, WHO, EMRO, Cairo, Egypt for their assistance and concern in publishing this report.

REFERENCES

Burke DS, Nisalak A, Ussery MA (1982): Antibody capture immunoassay detection of Japanese encephalitis virus immunoglobulin M and G antibodies in cerebrospinal fluid. Journal of Clinical Mircobiology 15:1034–1042.

Carey DE, Causey OR, Reddy S, Cooke AR (1971) Dengue viruses from febrile patients in Nigeria, 1964–1968. Lancet 1:105–106.

Clarke DH, Casals J (1958): Techniques for hemagglutination and hemagglutination inhibition with arthropod-borne viruses. American Journal of Tropical Medicine and Hygiene 7 561–573

Fagbami AH, Fabiyi A (1976). Epidemiology of Dengue infections in Nigeria. Virus isolations and clinical observations, 1972–1975. Journal of Tropical Medicine and Hygiene 1.226–229.

Gonzales JP, Du Saussay C, Gautun JC, McCormick JB, Mouchet J (1985). La Dengue au Burkina Faso (ex. Haute-Volta)· Epidemies saisonnières en milieu urbain a Ouagadougou. Bulletin de la Société de Pathologie Exotique et des ses Filiales 78.7–14.

Johnson BK, Ocheng D, Gichogo A, Okiro Mary, Libondo D, Kinyanjui P, Tukei PM (1982). Epidemic Dengue fever caused by Dengue type-2 virus in Kenya: Preliminary results of human virological and serological studies. East African Medical Journal 59.781–784.

Saleh AS, Hassan A, Scott RMcN, Mellick PW, Oldfield EC, III, Podgore JK (1985). Dengue in North East Africa Lancet 2·211–212.

Wulff H, Lang JV (1975): Indirect immunofluorescence for the diagnosis of Lassa fever infection. Bulletin of World Health Organization 52:429–436.

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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b RESTRICTIVE MARKINGS
2a SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; Distribution is unlimited.
2b DECLASSIFICATION/DOWNGRADING SCHEDULE	
4 PERFORMING ORGANIZATION REPORT NUMBER(S) 84/89-90	5 MONITORING ORGANIZATION REPORT NUMBER(S)
6a NAME OF PERFORMING ORGANIZATION U.S. Naval Medical Research Unit No. 3	6b OFFICE SYMBOL (If applicable) NAVMEDRSCHU THREE
6c ADDRESS (City, State, and ZIP Code) PSC 452, Box 5000 FPO, AE 09835-0007	7a NAME OF MONITORING ORGANIZATION 7b ADDRESS (City, State, and ZIP Code)
8a NAME OF FUNDING SPONSORING ORGANIZATION Naval Medical Research and Development Command	8b OFFICE SYMBOL (If applicable) NAVMEDRSCH DEVCOM
8c ADDRESS (City, State, and ZIP Code) National Naval Medical Center Building 1, Tower 12 Bethesda, MD 20889-5044	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER 10 SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO	PROJECT NO	TASK NO	WORK UNIT ACCESSION NO.
62770A	3M1627-70A870	AR	

11 TITLE (Include Security Classification)
Serological Evidence of Dengue Fever Among Refugees, Hargeysa, Somalia. (UNCLASSIFIED).

12 PERSONAL AUTHOR(S) Botros, Boulos A.M., Watts, Douglas M., Soliman, Atef K., Salib, Adel W., Moussa, Mahmoud I., Mursal, H.*, Douglas, C.* and Farah, M.*

13a TYPE OF REPORT	13b TIME COVERED FROM ____ TO ____	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
		1989, June, 04	3

16 SUPPLEMENTARY NOTATION
Published in: J. Med. Virol., 29:79-81, 1989; Acc. No. 1602.

17 COSATI CODES		
FIELD	GROUP	SUB-GROUP

18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Dengue; ELISA; IgM antibody; Patients; Hargeysa, Somalia.

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

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☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	UNCLASSIFIED

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Research Publications Branch	202-284-1381	R.P.B.

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted
All other editions are obsolete

SECURITY CLASSIFICATION OF THIS PAGE

☆ U.S. Government Printing Office: 1985—607-044

UNCLASSIFIED

19. have been the cause of the epidemic among the Dam Camp refugees.

12. * Refugee Health Unit, Ministry of Health, Mogadishu, Somalia.